Tissue regenerating agent

The invention relates to a tissue regenerating agent. The fields of application of this agent are medicine and the pharmaceutical industry.

A number of human tissues and organs essentially comprise terminally differentiated cells. These include, inter alia, the nerve cells of the brain and the cardiomyocytes of the heart. These cells are terminally differentiated, i.e. they no longer divide and cannot be induced to proliferate. This means that damaged or even dead cells cannot be replaced by proliferating, neighbouring cells, for example as in the healing of a wound. If, for example, in a blockage of the coronaries, cardiomyocytes are not sufficiently supplied with oxygen (heart infarct), the affected cells die off and are not replaced by new cardiomyocytes, but by fibrotic tissue, which can lead to drastic impairments of the function of the cardiac muscle. These coronary heart diseases are one of the most frequent diseases of the heart.

At present, there are no possibilities of therapy directly treating the causes, but merely attempts to limit the consequences of a heart attack. In severe cases, only a heart transplantation remains as the last resort. The objective of this invention is now to induce terminally differentiated cells to divide again, with the result that they can contribute to the regeneration of damaged, neighbouring tissue.

In principle, terminally differentiated cells can be induced to proliferate by tumour viruses. However, this unfortunately results in an irreversible transformation of the cells, i.e. a terminally differentiated and functioning cardiomyocyte is mutated into a cancer cell which grows uncontrolled, and in addition has lost the cardiac muscle

function. This approach is therefore not suited for therapeutic purposes.

Recently, a viral protein, VP22 from Herpes Simplex, has been described, which is exported from infected cells and taken up by neighbouring cells. The precise mechanism is not yet known. However, the transport process is independent of direct cell-to-cell contacts. Other proteins can also be transported when fused with the viral protein (Elliott G and O'Hare P (1997) Intracellular trafficking and protein delivery by a herpes virus structural protein. Cell 88: 223-233).

Anspruch 2

The objective of the invention is to provide a novel agent for the regeneration of tissue. It is based on the task of producing a fusion protein which induces the cells of damaged tissue to temporarily proliferate, thus effecting the regeneration of the tissue.

Anspruch 3

This task is solved in the measures portrayed in the claims.

The inventive tissue regenerating agent comprises an agent containing a fusion protein derived from a protein or a peptide sequence effecting the uptake in the cells, and a protein that induces the proliferation of cells. Preferably, a fusion protein derived from the viral VP22 protein with the SV40 T-antigen is used. On the basis of its varied functions, which cause cell proliferation and prevent apoptosis, the SV40 T-antigen is particularly well suited for this task. As an alternative, T-antigen related proteins or viral cyclins can be used. These cyclins include the K and V cyclins of the herpes virus. These cyclins are not inhibited by the cell cycle inhibitors of the cell and can thus induce proliferation without being impeded.

The second part of the task entails only temporarily inducing proliferation. After a few cell cycles, the cells are to return to the original terminally differentiated status and exercise their actual function. This task is solved with the inventive agent in that the agent is a protein which cannot replicate itself and is degraded by proteolytic enzymes. The stability of the fusion protein can be artificially amended as required by the inclusion of stabilising or destabilising peptides. This approach thus avoids irreversible genetic alterations and transformations of the cell, which would occur in DNA-based methods.

The use of this agent is done according to its purpose for regeneration of infarct-damaged cardiac tissue and for regeneration of nerve tissue damaged by injuries or disease. The agent is injected into the damaged areas and there taken up by the neighbouring cells. These cells are induced to proliferate, replace the dead cells and thus effect the tissue regeneration.

The agent is further also used according to its purpose for cultivation of terminally differentiated cells. Although terminally differentiated cells, e.g. nerve cells and cardiomyocytes, can be cultivated ex vivo, they do not proliferate and cannot be expanded for re-implantation or research purposes. The inventive agent is taken up by the cells following insertion into the culture medium and then effects the proliferation, i.e. multiplication of these cells. The dosage and duration of the treatment can be stipulated as required. After application of the agent has been stopped, the cells differentiate again and can either be re-implanted or used for research purposes.
It has been shown that the inventive agent can induce the S phase in terminally differentiated skeletal muscle cells (myotubes).

The invention is to be illustrated on the basis of a concrete example.

Example of implementation

The VP22 (UL49) gene is amplified with PCR from the Herpes Simplex Angelotti virus strain with primers which flank the open reading frame and remove the stop code. BamHI and XmaI restriction sites are added to the ends of these primers; with them, the PCR product can be cloned directly into an expression vector (pEVRF, Matthias et al., 1989). The T-antigen gene is amplified analogously from the SV40 DNA by means of PCR, and a XmaI and a XbaI restriction sites are added to the primers used. The PCR product is thus inserted into the expression vector at the C-terminal end of the VP22 gene. In the final cloning step, an oligonucleotide coding 6 histidine residues (His tag) and a stop codon is inserted at the C-terminal end of the T-antigen gene at the XbaI position. The final product is a fusion gene comprising the VP22 gene, the T-antigen and a His tag. The fusion gene is transcribed from the CMV promoter of the expression vector and translated from the translation signal of the Tk gene.
This expression vector is used to transfect COS-7 cells as described (Leonhardt et al., 1992). The fusion protein is exported from the producing cells into the medium due to the transport properties of VP22. The culture medium of the transfected COS cells conditioned in this way is continuously pumped via an affinity column (TALON, Clontech, Palo Alto, USA), which specifically binds the fusion proteins with a histidine tag. These affinity columns are used according to the instructions from the manufacturer. The fusion protein can then be eluted specifically with Imidazol and further purified by means of FPLC (ion exchange columns). The purified fusion protein is dialysed against normal saline solution and applied via catheters

directly into the cardiac muscle via the coronary arteries. As an alternative, the fusion protein can be injected directly and locally into the ischaemic cardiac muscle tissue.

Literature

Elliott G and O'Hare P (1997) Intercellular trafficking and protein delivery by a herpesvirus structural protein. Cell 88: 223-233

Leonhardt H, Page AW, Weier HU et al (1992) A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. Cell 71: 865-873

Matthias, P, Müller M M, Schreiber E, Rusconi, S and Schaffner, W. (1989) Eukaryotic expression vectors for the analysis of mutant proteins. Nucl. Acids Res. 17, 6418